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THE ORIGIN OF BILATERALITY IN VERTEBRATES[1]

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MANY attempts have been made to determine how early in development the vertebrate egg becomes bilaterally symmetrical. The conclusions have been as varied as the attempts.

Before the subject can be discussed it is necessary to consider two fundamental propositions. The first is that there exists an active pole in the egg, and the second is that the anterior end of the embryo develops in this region, or at least in the active hemisphere.

The active pole is indicated at an early period by certain phenomena, such as secretory activity, accelerated yolk metabolism, formation of pigment, position of nucleus, expulsion of polar bodies, etc. Hatschek says that "it is probable that a polar differentiation is present in the unfertilized ova of all the metazoa, through which the most active and least active poles can be determined." Whether or not Hatschek's statement be true, it is certain that if the area in which cleavage grooves first appear be traced backward a differentiation in this area can be found in a very early stage. We are thus enabled to speak of an active pole and an opposite inactive pole. A line passing through the two is designated as the primary ovic axis.

That the active pole or hemisphere gives rise to the embryo was first pointed out by Jan. Swammerdam in his "Bibel der Natur." This view was later supported by Prevost and Dumas, von Baer, Reichert, Cramer, Newport and others. Pflüger, however, believed that the greater portion of the embryo was formed from the inactive hemisphere and his view was supported by Roux, O. Hertwig and others. Most of the later investigators

[1] With observations by C. O. Whitman on Bufo.

including Morgan and Tsuda, Assheton, H. V. Wilson, King, Smith and others have generally agreed that the head end of the embryo forms from the active hemisphere and the caudal portion from the inactive. My own experiments on a considerable number of Amphibia have led to the conclusion that the head of the embryo forms from material which lies at, or near, the active pole of the egg. It thus seems fair to assume that the cephalic portion of the embryo is formed from the active hemisphere.

As stated there have been many attempts to determine how early in development the egg shows bilateral symmetry. Some claim bilateralism for the primitive ovum. Others hold that this condition is not present from the first, but originates at some later period. This period may precede or follow the deposition of the egg. Those who regard the egg as bilaterally symmetrical before deposition claim that this is manifested either through an excentric position of the egg nucleus, or an excentric pigmentation. Those who regard it as fixed after deposition are not in accord. By some the path of the spermatozoon is considered as the determining factor, by others the first or second cleavage groove, and by still others areas of accelerated segmentation.

The assumption that the egg is bilaterally symmetrical from the beginning is based upon nothing more than plausible hypothesis and naturally falls beyond the range of experimental proof.

Some (Schultze) hold that the excentric position of the egg nucleus together with the primary ovic axis determine bilaterality. The work by Roux, Jordan and others, shows that this is highly improbable.

Others (Roux, Morgan and Tsuda) maintain that the excentric arrangement of pigment enables one to determine bilaterality. Professor Whitman's observations which are recorded in a later paragraph, together with his drawings, indicate that the arrangement of the pigment is of significance in *Bufo*. The observations of Moskowski on *Rana*, Morgan's later observations on

Bufo, together with my own on *Amblystoma,* have thrown doubt upon this conclusion.

Still others (Newport, Roux) believe that the path of the entering spermatozoon and the primary ovic axis determine bilaterality. Jordan has shown that this view is untenable for *Diemyctylus.* Professor Whitman's observations, recorded in a later paragraph, show that this is not true in *Bufo.*

Thus each of these assumptions has been met by serious objections.

The idea that the first plane of cleavage determines the axis of the embryo was expressed as early as 1853 by Newport in the following words:

> I have long been aware that the axis of the embryo was in the line of the first cleft of the yolk.

From a series of experiments on the frog's egg Roux came to the conclusion that the first cleavage plane coincides with the median sagittal plane of the embryo. In the same year Pflüger reached the same conclusion. Supported by these eminent investigators the theory was very generally accepted. In working over the same field Rauber found that in the axolotl and frog the median plane of the embryo coincided with the second cleavage groove instead of the first. Shortly after the publication of Rauber's work, O. Hertwig working on the egg of *Triton* confirmed the observations of Rauber. In 1892 Roux modified his earlier view and stated that the second groove as well as the first often coincided with the median plane of the embryo.

In the following April the writer found from a series of puncture experiments on the egg of *Amblystoma* that exovates on opposite sides of the first cleavage groove were later found on one side of the embryo. The conclusion was that in these cases the first cleavage groove did not separate the right and left halves of the embryo.

In 1893 Jordan and the writer reviewed the experiments up to this date. We found that even in the descriptions and figures given by Newport, Roux, Rauber, there was evidence sufficient to show that the median plane of the embryo often deviated widely from the first or second cleavage planes. We accordingly undertook an extended series of observations on the living segmenting eggs of *Amblystoma, Diemyctylus, Rana* and *Bufo*. Our conclusions were as follows:

> The first and second cleavage planes undergo, even in the earlier stages, extensive torsion. Everything indicates that the extent of this shifting increases greatly in later stages. This led us to conclude that the earlier cleavage planes and the embryonic axes have no vital connection and that the coincidence where it exists is of no fundamental significance.

The later observations by Grönroos, v. Ebner, Morgan and Tsuda, Kopsch and others have likewise emphasized the significance of these variations.

It is scarcely necessary to state that if these cleavage planes mark embryonic areas, the amount of material set apart in different eggs for similar parts of their respective embryos, must be exceedingly variable, and these excesses and deficiencies must be corrected by a corresponding retarded or accelerated growth until the norm is reached, but there is not the slightest evidence that such corrections occur.

These wide variations have been repeatedly observed not only in various amphibia but also in practically all classes of vertebrates: in *Amphioxus* by Wilson; in *Petromyson* by McClure, Kupffer, Eycleshymer; in Dipnoans by Semon; in Ganoids by Salensky, Dean, Whitman and Eycleshymer; in Teleosts by Coste, Hoffmann, His, Agassiz and Whitman, Kingsley and Conn, Clapp, Sobotta and others; in Reptiles by Agassiz and Clark, Oppel, Sarasin; in Aves by Coste, Koelliker, Kionka; in Mammals by Duval, v. Beneden, Assheton, Sobotta and many others.

The inevitable conclusion from such a mass of evidence can not be other than that neither the position or direction of cleavage grooves has the slightest significance as far as the setting apart of definite embryonic areas is concerned.

If then it may be considered an established fact that neither the position nor the direction of the cleavage grooves enables one to predict the long axis of the embryo, we are naturally led to look for other phenomena which may be of significance. As stated in an earlier paragraph my experiments showed that the head end of the embryo is formed at, or very near, the active pole, and since this area is the one in which cell division is most rapid, it was concluded that the anterior end of the embryo, which is the first to differentiate, was indicated by this increased cellular activity. I accordingly stated that an area of increased cellular activity indicates the position of the head end of the embryo. As is well known, this area can be located with the advent of the first cleavage groove.

While the head end of the embryo may thus be readily located, the median plane of the body may lie in any one of an indefinite number of meridians. The question which now arises is which one of these meridians will represent the median plane of the future embryo.

The writer's studies on *Rana, Bufo, Acris, Amblystoma, Necturus* have shown that in another portion of the egg there is an area of smaller cells, and that this area of smaller cells always marked the region of the forthcoming blastopore. The blastopore in turn definitely fixes the posterior portion of the embryo.

With the recognition of these areas of accelerated cellular activity, the one at the active pole, indicating the position of the future head of the embryo, the other at the side of the egg, indicating the position of the forthcoming blastopore, it necessarily follows that the median plane of the embryo must coincide with a line passing through the centers of the two.

When these observations were first published in 1898, many questioned the existence of such a secondary area of cellular activity. Yet a search through the literature showed that such an area had been observed in many groups of vertebrates. Lwoff found such an area at the posterior end of the embryo of *Amphioxus*. The figures of the segmenting blastodiscs of Elasmobranchs, given by Balfour, Rückert, Gerbe and Sobotta all show that in these forms such an area is present. In the Reptilia, Vay's studies on *Tropidonotus* show that an area of small cells represents the posterior end of the embryo. v. Koelliker first called attention to such an area in the blastodisc of the chick and suggested that it determines the position of the posterior end of the embryo. The later investigations of Duval and Kionka leave no doubt as to the frequent and probably constant appearance of this area in the locality which later becomes the posterior end of the embryo.

In 1904 the writer made a study of the egg of *Necturus,* which from its size is especially favorable for surface study. This work was undertaken with a view of ascertaining how early this secondary area could be located. It was found that as early as the fourth or fifth cleavage, the cells on one side began to divide more rapidly than any others, excepting those of the primary area. It was possible to predict in this form the median plane of the forthcoming embryo at an extremely early stage of cleavage.

The following year de Bussy from his studies on the Japanese *Cryptobranchus* emphasized the fact that he could find no secondary area of accelerated cell division such as had been described by the present writer. Yet Smith working on the American *Cryptobranchus* says that he finds "an accelerated cell division about a radius of the blastodisc which gives a condition of bilateral symmetry."

The writer felt that it was scarcely necessary to follow the subject further and should not have rehearsed the findings had it not been that certain material came into his hands last year which bears directly upon this subject. This material consists of unpublished descriptions and drawings made by the late Professor C. O. Whitman in June, 1894. These were turned over to me by the department of zoology of the University of Chicago. Professor Whitman's notes run as follows:

Hitherto we have obtained eggs the first week in June. This year we could find none until July 1. We had several night rains, enough to flood the low ground behind Breakwater Hotel. On the evening of June 30, the day after the rain fell copiously, the toads swarmed in this place, and had a carnival of noise; the whole place rang with so many voices as to be almost deafening. On the morning of July 1, we found a great many eggs. The following night the singing followed but much reduced, and only two pairs of toads were captured. The next night the water had gone except in one of the ditches and no toads were to be heard, and of course no eggs. It would seem that rains stimulate them to lay; and the lateness of the season may have been the reason that the egg-laying was confined almost entirely to a single night.

The unfertilized eggs are by some said to be unoriented, that is they are said to be unable to take the normal position assumed by the fertilized egg. The sperm is supposed by some observers to mark the first plane of division and to give the egg the power to right itself. I find that it is not true that the eggs will lie just as they happen to fall, although they do so more nearly before fertilization than after it. If an egg be separated from the rest and turned about for some moments with needles, so as to loosen its adhesion to the membrane, and then rolled to one side so that the equator is vertical, one observes that it slowly turns and in the course of a minute, or sooner, it takes the normal position with the blacker pole uppermost and the whiter showing a little on one side, when viewed from above. This was repeated several times and on several eggs with the same result. The motion is so slow that one does not notice it until after the lapse of some seconds.

I cannot affirm that all unfertilized eggs will right themselves; ordinarily they do not if left to themselves. They assume an irregular wrinkled appearance and have so little power of righting that they stick to the membrane enough to prevent it. When fertilized they contract and round up and get freedom of space to move in. The entrance of the sperm evidently increases the disproportions between the weights of the upper and lower pole. The upper pole becomes lighter and the egg rights itself more readily and quickly. The orientation of the egg is complete before fertilization.

In the eggs which are in the stage of first cleavage there is a small depression which I have found by examination of earlier stages is the "fovea germinativa" of Max Schultze, or the "fosette germinative" of Bambeke. I find further after fertilization, a second point or depression, which probably is the place of penetration of the spermatozoon. The fovea marks the upper pole, but is not placed at the middle of the upper hemisphere; it is excentric.

I followed two eggs which showed both the fovea and the spermatic dent. In neither did the first cleavage plane pass through this dent. In one case it passed far from it while the second cleavage passed near to it. In another case the dent is in the middle of one of the first four cells, and on the darker side of the upper hemisphere. If this be the sperm track it does not determine the median plane of the embryo.

CLEAVAGE

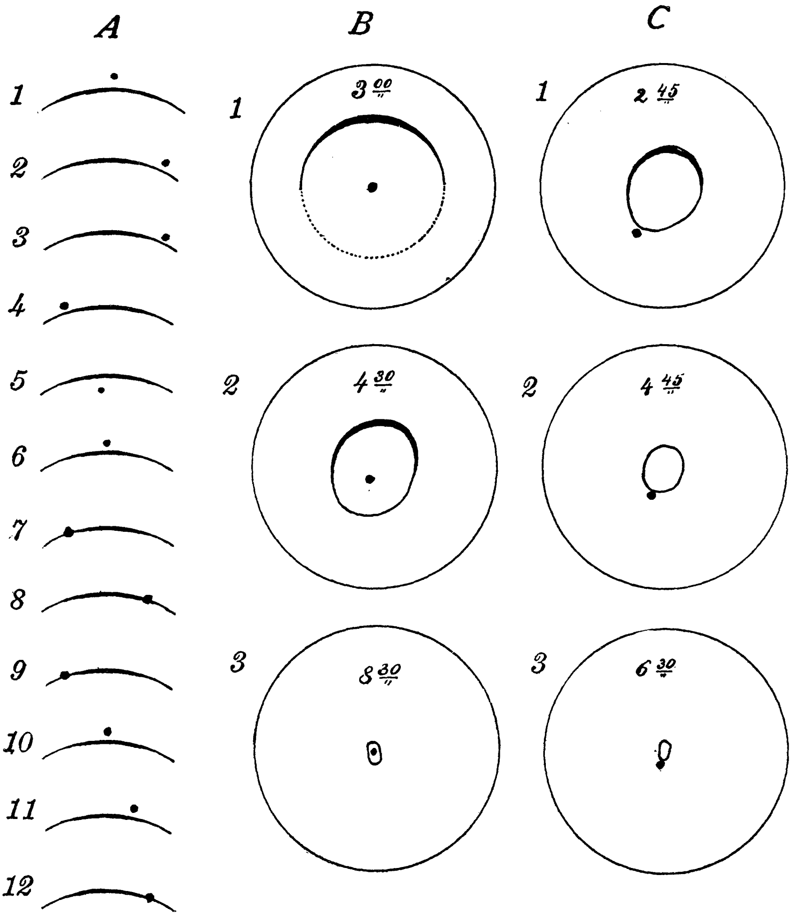
The eggs were obtained in the two-cell and four-cell stages. At this time the pigment is excentric, falling a little short of the equator on the one side and a little beyond it on the opposite. [The notes nowhere state that the antero-posterior direction of the embryo is indicated by the distribution of pigment, yet I think an examination of the figures can not fail to convince all that their interpretation can not be otherwise.—A. C. E.] When the first cleavage groove runs in the plane of symmetry the second cleavage grooves are at right angles and appear at about the same time in both halves as shown in Figs. 3 and 4. When the first cleavage groove is transverse to the plane of symmetry the second cleavage grooves do not appear at the same time, but the one on the lighter side of the upper hemisphere appears first, as shown in Fig. 1. The third cleavage usually cuts off all the pigment into the upper cells on the blastoporic (posterior) side, but leaves considerable below the upper cells on the opposite (anterior) side. The second equatorial usually cuts off all the pigmented cells on the anterior side of the egg and non-pigmented cells on the posterior (blastoporic) side.

The blastomeres on the posterior (blastoporic) side are smaller than on the anterior side, from the very first. It is the blastoporic side that takes the lead in division and the cells are smaller here all the way up to the time when the blastopore appears.

It is thus obvious that the findings by Professor Whitman not only lend confirmation to my observations on bilaterality, but that they in reality anticipate them.

It may be said with added confidence that bilaterality in the vertebrate egg is revealed through the early cleavage grooves. The cephalic portion of the embryo is indicated by the area in which cleavage grooves first appear and in which cellular division is most rapid. The caudal portion is indicated by a secondary area of cellular activity in the blastoporic region. These two areas pass into each other constituting an embryonic tract.

In addition to the above observations, Professor Whitman's manuscript and drawings give the results of a series of puncture experiments in the blastoporic lip. Since these observations have an important bearing on the question of epiboly, emboly and concrescence, they are appended.


A
B
C
3 00
4 30
8 30
2 45
4 45
6 30

EXPERIMENTS

On June 5, 1894, sixteen eggs in the thirty-two cell stage were punctured at the equator, in the middle of the white cells, as shown in Fig. 11. In twelve the blastopore appeared near the puncture as shown in the accompanying cut. The extraovates were found in the positions shown in *A*, 1–12, at 10:00 A.M. the next morning. The variations in positions are doubtless due to my punctures falling at different points, sometimes hitting as in Fig. 12, at other times in the very edge of the pigment. In the four remaining eggs two showed no extraovate and two showed no blastopore.

On June 4, 1894, pricked egg *B* at middle of lower pole, soon after the blastopore was sharply marked on the side of the embryo. Ventrally this outline was not clearly marked. At 4:30 this blastopore was outlined all around and nearly circular or about ½ diameter observed at 3:00. At 6:30 the blastopore was far advanced and nearly circular. At 8:30 it was nearly closed. It will be noted that the extraovate remained central throughout.

Another egg *C* was punctured in the ventral edge of the blastoporic rim, and the extraovate was carried along by the closing blastopore. I ought to have made two punctures, one in the middle as well, so that this approach could have been seen. However, my notes show that the blastopore advanced evenly. In this case the extraovate is carried along by this overgrowth, and one might imagine this puncture a fixed point, approached by the blastopore from the opposite side.

June 5. I pricked a number of eggs in the early cleavage stages (8–64 cells) at lower pole. In most of these eggs the extraovates were found after two to three hours to lie at or near the equator of the egg. This was long before the appearance of the blastopore. The extraovate has evidently moved and if one should leave the egg until the blastopore appeared and then look at it, it might be found at the middle of the body; and thus it might appear as if the embryo had lengthened across the lower pole (Roux). Sometimes extraovates have moved and the punctures healed.

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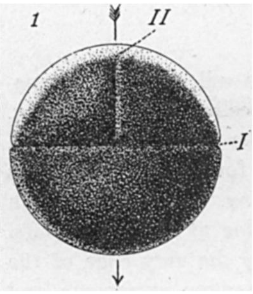

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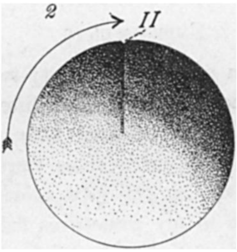

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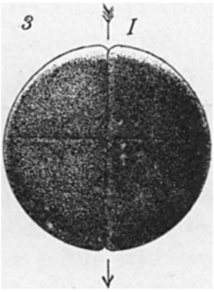

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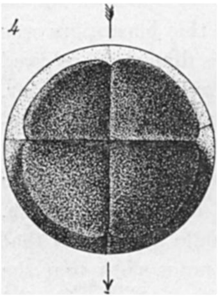

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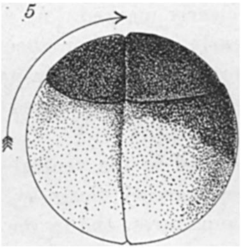

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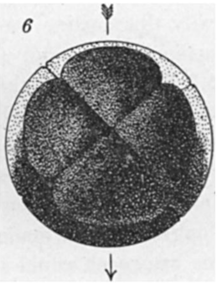

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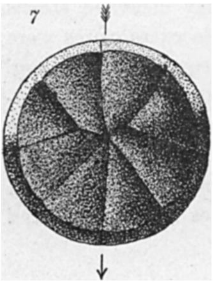

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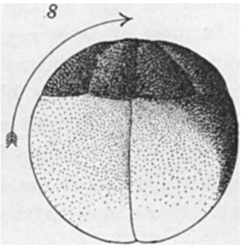

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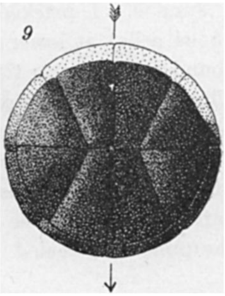

EXPLANATION OF PLATES

Since no explanation of the figures could be found other than those included in the preceding pages, I have endeavored to give an explanation in accord with the text. It should be remembered however that the figures may be open to other interpretations than those presented. The figures show the distribution of pigment and the relation of the embryo and the cleavage planes to the pigment. It will be noted that the eggs when viewed from above show a lighter area or crescent on one side. This excentric position of the pigment is likewise well shown in profile. The arrows in all cases show the direction of the forthcoming embryo.

FIG. 1. Shows the upper hemisphere of an egg in which the embryonic axis is indicated by a line passing through the centers of the light crescent and the more deeply pigmented area. In this case the first cleavage plane (I) passed at right angles to the embryonic axis. It is of interest to note that the second cleavage (II) has appeared in that portion of the egg nearest the grey crescent and further that it coincides with the median plane of the embryo.

FIG. 2. Shows a profile view of an egg in which the first cleavage coincides with the median plane of the embryo while the second is at right angles to the same. I am at a loss to understand the extent of the arrow in this and the succeeding profile views. It may be that Professor Whitman intended thus to indicate the limits of the embryonic anlage.

FIG. 3. Shows the upper hemisphere of an egg in the four cell stage. In this case the median plane of the forthcoming embryo coincides with the first cleavage groove.

FIG. 4. Shows the upper hemisphere of an egg consisting of eight cells. It is to be noted that the formation of the first equatorial sharply separates the lighter and darker portions of the egg on the one side but not on the opposite side. In this case the median plane of the embryo coincides with either the first or second cleavage groove.

FIG. 5. Represents the profile view of either the same egg or another egg in the same stage. In this case the differences in the distribution of the pigment are again shown.

FIG. 6. Shows the upper hemisphere of an egg in which neither the first nor the second cleavage grooves coincide with the median plane of the embryo.

FIG. 7. Shows the upper hemisphere of an egg at a time when the fourth cleavage grooves are present. It is impossible to say whether the median plane of the embryo coincides with either the first or the second cleavages. The appearance of the cleavage grooves leads me to infer that the direction of the arrow is parallel with either the first or the second.

FIG. 8. Represents a profile view of either the same egg or another egg in the same stage.

FIG. 9. Shows the upper hemisphere of another egg in which the fourth cleavage grooves are present. In this egg the median plane of the embryo coincides with the first or second cleavage groove.

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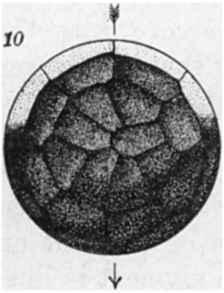

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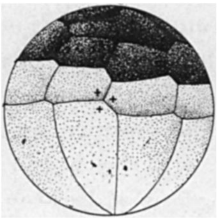

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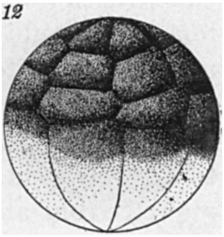

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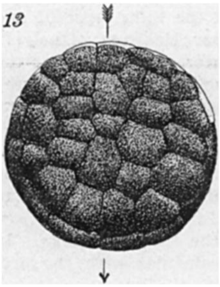

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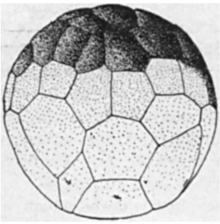

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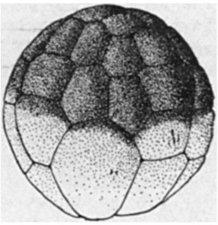

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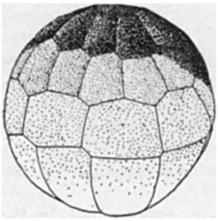

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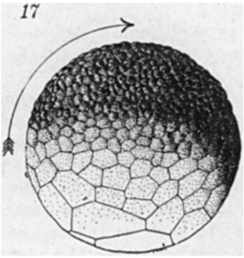

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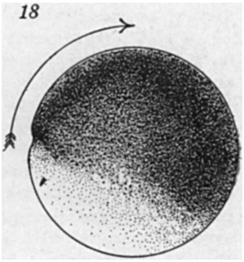

FIG. 10. Shows the upper hemisphere of an egg in a later stage of cleavage. It should be noted that the lines representing the primary grooves are entirely obscured by a shifting of the blastomeres.

FIG. 11. Represents a profile view of the same (?) egg, viewed from the side on which the blastopore is forthcoming. The small crossed lines represent the localities in which Professor Whitman punctured the eggs of this stage.

FIG. 12. Represents a profile of the opposite side of the same (?) egg.

FIG. 13. Shows the upper hemisphere of an egg in a later stage of cleavage. It should again be emphasized that it would be impossible to trace any one of the primary cleavage grooves. The cells have undergone shiftings to such a degree that if the median plane of the embryo coincided with either of the first two grooves it must be extremely irregular.

FIG. 14. Represents a profile view of the same (?) egg viewed from the side in which the blastopore will later appear. On this side cell division is decidedly in advance of the opposite side.

FIG. 15. Represents a profile view of the opposite side of the same (?) egg.

FIG. 16. ?

FIG. 17. Represents a profile view of an egg in late segmentation. The side in which the blastopore will appear is now indicated not only by the distribution of pigment but also by a decided acceleration in cell division.

FIG. 18. Represents a profile view of an egg at the time when the blastopore appears. The figure shows that it appears on the side of the egg which is least pigmented.